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(54) ANTIBIOTIC B—41

(71) We, SANKYO COMPANY LIMITED, a Japanese Body Corporate, of 1-6, 3-chome, Nihonbashi Honcho, Chuo-ku, Tokyo, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to new antibiotic B—41, to its production by culturing an antibiotic B—41-producing strain belong in the genus *Streptomyces*, and to an insecticidal and acaricidal composition containing novel antibiotic B—41 or a constituent thereof as an active ingredient.

Many organic compounds have heretofore been used as insecticidal and acaricidal preparations. Among antibiotic substances, however, only a few substances have been known to have insecticidal and acaricidal effects. Moreover, they have not been put into practical use yet.

As the result of extensive studies, we have found that a novel antibiotic substance B—41, which is produced by a new strain belonging to the genus *Streptomyces* (the "B—41—146 strain" Bikokenkinki No. 1438) is not only far higher in acaricidal activity than known organic compounds having acaricidal activities but also is effective for the control of agriculturally and horticulturally harmful insects such as aphids and larvae of insects of the order *Lepidoptera*.

The antibiotic substance B—41, which is the active ingredient of the insecticidal and acaricidal compositions of the present invention, can be separated into 9 different constituents—*viz*, A_1, A_2, A_3, A_4, B_1, B_2, B_3, C_1 and C_2. Physicochemical properties of these constituents are as shown in Table 1.

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TABLE 1

			A_1	A_2	A_3	A_4	B_1	B_2	B_3	C_1	C_2
Molecular formula			$C_{32}H_{48}O_7$	$C_{38}H_{56}O_{10}$	$C_{31}H_{44}O_7$	$C_{32}H_{46}O_7$	$C_{39}H_{58}O_{10}$	$C_{32}H_{46}O_7$	$C_{33}H_{48}O_7$	$C_{36}H_{47}O_9N$	$C_{37}H_{49}O_9N$
Elementary analysis (%)	Calculated	C	70.56	67.83	70.43	70.82	68.19	70.82	71.19	C: 67.80	C: 68.18
		H	8.88	8.39	8.39	8.54	8.51	8.54	8.69	H: 7.43 N: 2.20	H: 7.58 N: 2.13
	Found	C	70.74	71.73	65.73	69.85	68.00	69.66	70.72	C: 65.93	C: 68.09
		H	9.18	8.26	7.89	8.37	8.32	8.32	8.59	H: 7.53 N: 2.14	H: 7.54 N: 2.13
Molecular weight	Osmometric method (in acetone)		513.9	672.1	517.0	–	629.5	524.3	–	–	–
	Mass spectrum (M+)		544	672	528	542	686	542	556	679 (Note 1)	693 (Note 1)
Melting point (°C.)			Amorphous powder	Amorphous powder	212–215	193–195	176–178	139–142	Amorphous powder	Amorphous powder	Amorphous powder
Specific rotatory power $[\alpha]_D^{20}$ (Concentration of sample 5 mg/2 ml, length of layer in acetone 10 cm)			+160°	+54°	+106°	+103°	+75°	+131°	+126°	+57°	+54°

TABLE 1 (Continued)

	A_1	A_2	A_3	A_4	B_1	B_2	B_3	C_1	C_2
Molecular formula	$C_{32}H_{48}O_7$	$C_{38}H_{56}O_{10}$	$C_{31}H_{44}O_7$	$C_{32}H_{46}O_7$	$C_{39}H_{58}O_{10}$	$C_{32}H_{46}O_7$	$C_{33}H_{48}O_7$	$C_{36}H_{47}O_9N$	$C_{37}H_{49}O_9N$
Ultraviolet absorption spectrum (λ-max, mμ)	240.5 Fig. 1	245 Fig. 2	245 Fig. 3	245 Fig. 4	245 Fig. 5	245 Fig. 6	245 Fig. 7	240 Fig. 8	240 Fig. 9
Infrared absorption spectrum (Nujol method)	Fig. 10	Fig. 11	Fig. 12	Fig. 13	Fig. 14	Fig. 15	Fig. 16	Fig. 17	Fig. 18
Nuclear magnetic resonance spectrum [in $(CD_3)_2CO$ in the case of Figs. 19 to 25, and in $CDCl_3$ in the case of Figs. 26 and 27; 60 MHz]	Fig. 19	Fig. 20	Fig. 21	Fig. 22	Fig. 23	Fig. 24	Fig. 25	Fig. 26	Fig. 27
Mass spectrum (Main peaks under the conditions of 75 eV, ionization room temperature 200°C. and sample temperature 120° to 190°C.)	544 402 181 153	672 181 153 151	528 400 181 153 151	542 414 195 167 151	686 414 195 167 151 125	542 400 181 153 151	556 414 195 167 151	679 568 400 181 153 111 (Note 1)	692 582 414 195 167 111 (Note 1)
Solubility in solvents	Difficultly soluble in water; easily soluble in n-hexane, benzene, acetone, ethanol and chloroform	—do—	—do—	—do—	—do—	—do—	—do—	—do—	

TABLE 1 (Continued)

		A_1	A_2	A_3	A_4	B_1	B_2	B_3	C_1	C_2
Molecular formula		$C_{32}H_{48}O_7$	$C_{38}H_{56}O_{10}$	$C_{31}H_{44}O_7$	$C_{32}H_{46}O_7$	$C_{39}H_{58}O_{10}$	$C_{32}H_{46}O_7$	$C_{33}H_{48}O_7$	$C_3\ H_{47}O_9N$	$C_{37}H_{49}O_9N$
Colour reaction	Iodine/chloroform	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
	Ninhydrin	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
(by thin layer chromatography)	Sulfuric acid spraying with heating	Brown	Reddish purple	Brown	Brown	Reddish purple	Brown	Brown	Brown	Brown
	Potassium permanganate solution	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Alcohol solution, neutralization titration method		No pKa at pH ranging from 2 to 12	—do—	—do—	—do—	—do—	No pKa at pH ranging from 2 to 9 (crystallization took place at about pH 8.7 to make the measurement impossible)	—do—	No pKa at pH ranging from 2 to 12	—do—
Colour of substance		Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless

Note 1: The mass spectra for C_1 and C_2 were measured on the acetylated derivatives obtained by substitution of a CH_3CO-group for the hydrogen atom of R^1 in the plane structural formula (I) shown below.

The accompanying drawings show ultraviolet absorption, infrared absorption and nuclear magnetic resonance spectra of the antibiotic B—41. Figs. 1 to 9 show, respectively, the ultraviolet absorption spectra of its constituents A_1, A_2, A_3, A_4, B_1, B_2, B_3, C_1 and C_2; Figs. 10 to 18 show, respectively, the infrared absorption spectra of its constituents A_1, A_2, A_3, A_4, B_1, B_2, B_3, C_1 and C_2; and Figs. 19 to 27 show, respectively, the nuclear magnetic resonance spectra of its constituents A_1, A_2, A_3, A_4, B_2, B_3, C_1 and C_2.

The Rf values of the above-mentioned constituents were measured by thin layer chromatography using a thin layer chromatographic spot film containing a fluorescence reagent (available from Tokyo Kasei Kogyo Co. Ltd.: Trade name, " SPOT-FILM fluorescent ") and are shown in tables 2 and 3. The constituents were detected by the intensity of fluorescence emitted when each substance was irradiated with ultra-violet rays of 2536 Å.

TABLE 2

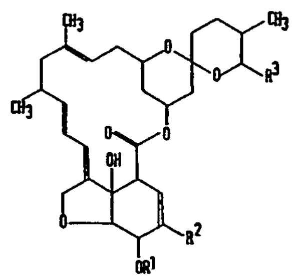
Silica gel F

Solvent system	A_1	A_2	A_3	A_4	B_1	B_2	B_3	C_1	C_2
Acetone/n-Hexane (30:70)	0.47	0.32	0.42	0.44	0.47	0.61	0.63	0.22	0.24
Ethyl acetate/Benzene (50:50)	0.61	0.62	0.63	0.65	0.79	0.80	0.82	0.45	0.47
Ethyl acetate/Chloroform (25:75)	0.27	0.35	0.39	0.41	0.75	0.79	0.81	0.12	0.13
Acetone/Benzene (40:60)	0.85	0.75	0.77	0.79	0.92	0.92	0.92	0.60	0.63
Acetone/Benzene (15:75)	0.39	0.32	0.39	0.39	0.57	0.51	0.53	0.11	0.13
Ethanol/n-Hexane (2:98)	0	0	0	0	0	0	0	0	0

TABLE 3
Alumina F

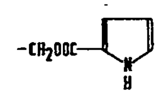
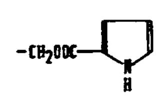
Solvent system	A_1	A_2	A_3	A_4	B_1	B_2	B_3	C_1	C_2
Acetone/n-Hexane (30:70)	0.55	0.16	0.32	0.34	0.67	0.92	0.92	0	0
Ethyl acetate/Benzene (50:50)	0.56	0.10	0.21	0.23	0.73	0.81	0.83	0	0
Ethyl acetate/Chlorofom (25:75)	0.40	0.10	0.15	0.17	0.63	0.65	0.67	0	0
Acetone/Benzene (15:75)	0.27	0.05	0.09	0.11	0.35	0.45	0.47	0	0
Ethanol/n-Hexane (2:98)	0.17	0.03	0.07	0.09	0.20	0.42	0.44	0	0

From the above-mentioned physicochemical properties, particularly the high resolution mass spectra, and from the results of X-ray analysis, it has been established that constituents A_3, A_4, B_2, B_3, C_1 and C_2 of antibiotic B—41 have the following plane structural formula:

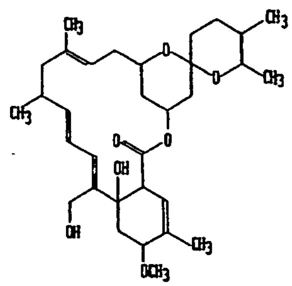


(I)

wherein R^1, R^2 and R^3 are as follows:—

	R^1	R^2	R^3
A_3	H	CH_3	CH_3
A_4	H	CH_3	C_2H_5
B_2	CH_3	CH_3	CH_3
B_3	CH_3	CH_3	C_2H_5
C_1	H		CH_3
C_2	H		C_2H_5

Likewise, it has been established that constituent A_1 of antibiotic B—41 has the following plane structural formula:



(II)

The structures of constituents A_2 and B_1 of antibiotic B—41 are not clear. From their mass spectra, however, it is inferred that they are similar in chemical structure to the above-mentioned constituents A_1, A_3, A_4, B_2, B_3, C_1 and C_2.

Since there are no known antibiotic substances having the aforesaid chemical structures and physicochemical properties, it has been established that the antibiotic B—41 of the present invention is a novel antibiotic substance.

The B—41—146 strain of the genus *Streptomyces*, which produces the antibiotic substance B—41, has the following mycological properties:

1) *Morphological characteristics:*

On most common laboratory media, long, aerial mycelium developed from fine branched substrate mycelium and formed whorls with spirals or loops.

Fragmentations of mycelium not observed at early stage.

Spores more or less short warty, 0.6—0.9 × 1.1—1.5 μ, formed in chains with 10—50 conidia.

Relatively short warty extrusions on surfaces of spores.

Sporangia and sclerotia not observed.

2) *Cultural characteristics on various media:*

i) Sucrose-nitrate agar:
Good growth; substrate mycelium colourless; reverse pale-brown; aerial mycelium scant, semi-transparent, coriaceous; soluble pigment pale-brown.

ii) Glucose-asparagine agar:
Abundant growth; substrate mycelium colourless; reverse pale-brown; aerial mycelium abundant, grey-coloured; soluble pigment pale-brown.

iii) Glycerol-asparagine agar:
Abundant growth; substrate mycelium colourless; reverse pale-brown; aerial mycelium white, and on slant, many bright greyish brown dots formed in white background; soluble pigment yellowish grey.

iv) Inorganic salts-starch agar:
Abundant growth, substrate mycelium colourless; reverse yellowish grey; aerial mycelium grey, and on slant, many pale yellowish dots formed in grey background; soluble pigment bright olive grey.

v) Tyrosine agar:
Abundant growth; substrate mycelium greyish yellow brown; reverse brown; aerial mycelium grey, and on slant, yellowish grey dots formed sometimes in grey background; soluble pigment bright brown.

vi) Nutrient agar:
Poor growth; substrate mycelium colourless; reverse pale-brown; aerial mycelium scant, white, soluble pigment not produced.

vii) Yeast extract-malt extract agar:
Abundant growth; substrate mycelium greyish yellow brown; reverse yellowish brown; aerial mycelium abundant, gery and on slant, many pale yellow dots formed in grey background; soluble pigment yellow.

viii) Oatmeal agar:
Abundant growth; substrate mycelium colourless; reverse olive grey; aerial mycelium grey, and on slant, pale yellow dots formed; soluble pigment pale olive.

3) *Physiological characteristics:*

i) Growth temperature range: 18°—37° C.
Optimum growth temperature: 25°—30° C.

ii) Liquefaction of gelatin: Slow but strongly positive.

iii) Hydrolysis of starch: Strongly positive.

iv) Coagulation of skim milk: Positive (28° C). Peptonization of skim milk: Positive (28° C)

v) Melanin formation: Negative.

vi) Reduction of nitrate: Positive.

vii) Utilization of various carbon sources (Pridham and Gottlieb agar)
Utilization degree:
*** Raffinose.
** D-Glucose, D-Fructose, Sucrose, L-Rhamnose, I-Inositol, D-Mannitol.
* L-Arabinose, D-Xylose.

From the above characteristics, this strain is most closely related to *Streptomyces chattanoogensis* (International Journal of Systematic Bacteriology, Vol. 18, No. 2, page 97 (1968)), but the latter differs from B—41—146 strain as follows:

(1) The aerial mycelium of the B—41—146 strain forms abundant whorls, whereas that of *S. chattanoogensis* is monopodially branched.

(2) The spore surface of the B—41—146 strain is warty, whereas that of *S. chattanoogensis* is spiny.

(3) On yeast-malt extract agar and inorganic salt-starch agar, the B—41—146 strain forms pale-yellow dots in a grey background, but *S. chattanoogensis* does not.

(4) The B—41—146 strain assimilates L-arabinose, D-xylose and I-inositol, whereas *S. chattanoogensis* does not assimilate these carbon sources.

In view of the above-mentioned 4 differences in mycological properties, we judged that the B—41—146 strain is a new species of the genus *Streptomyces*. The B—41—146 strain has been deposited at the Research Institute of Industrial Technology of Microorganisms, Agency of Industrial Science and Technology in Japan, with the deposition number *Bikokenkinki* No. 1438.

As is well known, *Streptomyces* tend to mutate both naturally and by application of such artificial operations as, for example, ultraviolet irradiation, ionizing irradiation, or chemical treatment. This is also the case with the B—41—146 strain used in the present invention. Consequently, any antibiotic B—41-producing mutant of the described *Streptomyces* strain B—41—146 may be used to produce the antibiotic B—41 of the invention.

In the process of the present invention, the antibiotic substances B—41 are obtained by culturing the B—41—146 strain in an aqueous nutrient medium, and then optionally recovering the resulting antibiotic B—41 from the fermentation broth. The strain may be cultured in stationary culture, but if it is desired to produce large quantities of antibiotic B—41 it is most preferable to culture the strain in liquid culture with aeration and agitation.

As the culture media, there may be used any which are ordinarily used for the culture of species belonging to the genus *Streptomyces*. Examples of suitable carbon sources include starch, dextrin, glucose, maltose, corn steep liquor and molasses, and examples of suitable nitrogen sources include meat extract, peptone, yeast extract, soybean meal, casein, ammonium sulfate and ammonium nitrate. If necessary, there may be added potassium, calcium, magnesium, iron, copper, zinc, manganese, cobalt and the like inorganic salts, or trace elements.

The antibiotic B41 can be recovered from the broth, by means of *per se* known techniques such as extraction with an organic solvent in the presence or absence of an adsorbent or auxiliary agent. For example, the cells may be separated by filtration from the broth and then extracted with an organic solvent such as methanol or acetone, or the broth may directly be subjected to extraction with an organic solvent such as chloroform, ethyl acetate, benzene, n-hexane or cyclohexane.

If desired, the oily crude B—41 obtained by removing the solvent from the extract can be purified by means of *per se* known purification procedures such as column chromatography or extraction with a solvent.

The invention is illustrated by the following Examples. In the Examples, in order to evaluate the activity of the broth, kidneybean leaves parasitized with two-spotted spider mites were dipped for 1 minute in a 70% acetone extract of the broth or in an aqueous dilution thereof and then air-dried, and the acaricidal activity was measured after 24 hours.

Example 1.

30 Litres of an aqueous culture medium (pH of about 7.2) containing 2.0% of glucose, 1.0% of soybean meal and 0.2% of sodium chloride were charged into a 50 litre-jar fermenter, and then sterilized by heating. The B—41—146 strain (Bikokenkinki No. 1438) was inoculated in the said medium and subjected to aerobic stirred culture at a temperature of 28° C., aeration at 8 litres/min. and agitation at 250 r.p.m. After cultivation for 120 hours, the broth was bright yellow. At this stage, the cultivation was discontinued, and the activity was examined. A 300 times dilution of the broth showed an acaricidal activity of 100%. Subsequently, the cells were separated by filtration from the broth and extracted with acetone, and then the acetone was removed by distillation by obtain 44 g. of a brown substance. This substance was extracted with hot hexane, and then the hexane was removed by distillation. The residue was dissolved in a small amount of methanol, and the resulting solution was allowed to stand overnight at −20° C. to deposit precipitates, which were then removed. Thereafter, the methanol was removed by distillation to obtain 35 g. of a brown oily substance. The thus obtained oily substance was subjected to alumina column chromatography and eluted with chloroform, effective fractions were collected according to acaricidal activity, and then the chloroform eluate was concentrated. This operation was repeated several times to obtain 8.2 g. of a crude substance. The acaricidal activity of the crude substance was 100% when used at a concentration of 2 μg/ml.

The crude substance was passed through a column packed with "Sephadex LH-20" (trade name for a product of Pharmacia Co.) and eluted with methanol, whereby A_2, B_1, B_2, A_3 and A_1, which are the main component of antibiotic B—41, were eluted in this order. However, these substances had overlapped with each other and hence were separately recovered in the form of 2 groups of $B_1 + A_2$ and $B_2 + A_1 + A_3$. The $B_1 + A_2$ group was subjected to silica gel column chromatography and then eluted with chloroform-ethyl acetate to obtain 210 mg. of B_1 and 115 mg. of A_2,

and the $B_2 + A_1 + A_3$ group was subjected to silica gel column chromatography to obtain 200 mg. of B_2 and 30 mg. of B_3, which was similar to B_2. Subsequently, the remaining $A_1 + A_3$ group was subjected to alumina column chromatography to obtain 372 mg. of A_1, 42 mg. of A_3 and 15 mg. of A_4, which was similar to A_3. Further, the alumina column, through which the aforesaid brown oily substance had been passed, was subjected several times to methanol elution and silica gel column chromatography to obtain 78 mg. of constituent C_1 of antibiotic B—41 and 52 mg. of constituent C_2 of antibiotic B—41.

For preparation of the insecticidal and acaricidal composition of the present invention, one or more of the thus obtained constituents A_1, A_2, A_3, A_4, B_1, B_2, B_3, C_1 and C_2 of antibiotic B—41 are diluted with a carrier and, if necessary, incorporated with other auxiliary agents, whereby the said substances can be formulated into compositions such as dusts, granules, fine granules, wettable powders, emulsifiable concentrates, or oil sprays. The purification may optionally be discontinued at any stage and the resulting crude product, which has not been completely purified, may be used as the active ingredient: for such use, it is sufficient that the crude product be purified so as to attain an acaricidal activity of 100% at the concentration of 5 p.p.m. In this case, the content of antibiotic B—41 in the crude product is about 50%, the remainder being impurities from the broth.

The carrier referred to herein means a synthetic or natural inorganic or organic substance which is added to an insecticide in order to make it easier for the active ingredient to reach objectives such as plants, mites, harmful insects, etc., or to facilitate the storage, transportation or handling of the active ingredient.

Examples of suitable solid carriers include inorganic substances such as clay, talc, diatomaceous earth, kaolin, bentonite, calcium carbonate and synthetic calcium silicate, natural and synthetic resins such as coumarone resins, alkyd resins and polyvinyl chloride; waxes such as carnauba wax and paraffin wax; shells of nuts such as walnuts and coconuts; and soybean flour.

Examples of suitable liquid carriers include water; alcohols such as ethanol, isopropanol and ethylene glycol; glycol ethers such as ethylene glycol monophenyl ether, and diethylene glycol monoethyl ether; ketones such as acetone, methyl isobutyl ketone, cyclohexanone, acetophenone and isophorone; ethers such as tetrahydrofuran and dioxane; aromatic hydrocarbons such as benzene, toluene, xylene and methyl naphthalene; chlorinated hydrocarbons such as trichloroethylene and carbon tetrachloride; and low, medium and high boiling petroleum fractions containing kerosine, light oils or aromatic hydrocarbons.

Examples of suitable propellants include Freon gases ("Freon" is a Trade Mark), liquefied petroleum gases, methyl ether and vinyl chloride monomer.

For emulsifying, dispersing, wetting or spreading, ionic or nonionic surface active agents can be used in the composition of the invention. Examples of suitable anionic surface active agents include the sodium and calcium salts of lignosulfonic acid, the sodium and potassium salts of oleic acid, the sodium salt of lauryl-sulfonic acid, and the sodium and calcium salts of dodecylbenzenesulfonic acid. Examples of suitable cationic surface active agents include higher aliphatic amines and ethylene oxide condensates of higher aliphatic amines. Examples of suitable nonionic surface active agents include glycerides of fatty acids, sucrose esters of fatty acids, ethylene oxide condensates of higher aliphatic alcohols, ethylene oxide condensates of higher fatty acids, ethylene oxide condensates of alkyl phenols and alkyl naphthols, and copolymers of ethylene oxide with propylene oxide.

The insecticidal and acaricidal composition of the present invention may contain a protective colloid such as gelatin, gum arabic, casein, polyvinyl alcohol or carboxymethyl cellulose, or a thixotropic agent such as sodium polyphosphate or bentonite. The composition of the present invention may further contain other compounds having insecticidal and acaricidal activities such as, for example, 2 - (1 - methylpropyl) - 4,6 - dinitrophenyl-β,β - dimethyl acrylate, di - (p - chlorophenyl) - cyclopropylcarbinol, N' - (2-methyl - 4 - chlorophenyl) - N,N - dimethylformamidine, 2,4,4',5 - tetrachlorodiphenylsulfone, 1,1 - bis(p - chlorophenyl) - 2,2,2-trichloroethanol, O,O - diethyl - S - (2-ethylthio)ethyl phosphorodithioate, O,O-dimethyl - S - (N - methyl - N - formylcarbamoylmethyl)phosphorodithioate, 2 - sec-butylphenyl - N - methylcarbamate or m-tolyl - N - methylcarbamate, or a mineral oil, whereby the effectiveness of the composition can be increased and, in some cases, synergistic effects may be obtained. The composition of the present invention may be used in admixture with fungicides, herbicides, plant growth regulators, attractants and fertilizers.

The insecticidal and acaricidal activity of compositions of the present invention is illustrated by Examples 2 to 6.

Example 2.

Emulsifiable concentrates containing 20% of each in turn of constituents A_1, A_2, A_3, A_4, B_1, B_2, B_3, C_1 and C_2 of antibiotic B—41, which had been isolated by the same pro-

cedure as in Example 1, were diluted to the concentrations shown in Table 4, to prepare test solutions. Kidneybean leaves infested with two-spotted spider mites were dipped for 1 minute in these test solutions and then air-dried, and the acaricidal activity (%) after 24 hours was calculated. The results obtained are shown in Table 4, which also shows, for comparison, results obtained with a known compound.

TABLE 4.

Concentration (p.p.m.) / Constituent	20	10	5	2.5	1.25	0.63	0.31	0.16	0.08	0.04
A_1	100	100	100	19.9	0					
A_2	100	100	100	67.6	0					
A_3	100	100	100	100	100	100	100	98.2	100	15.2
A_4	100	100	100	100	100	100	100	100	100	21.3
B_1	98.1	81.5	69.2	20.0	0					
B_2	100	100	100	100	100	100	80.5	16.6	0	
B_3	100	100	100	100	100	100	100	74.1	18.3	0
C_1	100	100	100	100	100	100	100	100	100	51.8
C_2	100	100	100	100	100	100	100	100	100	97.3
Reference*	100	56.4	0							

* 1,1-Bis-(*p*-chlorophenyl)-2,2,2-trichloroethanol (trade name, "Kelthane")

Example 3.

An emulsifiable concentrate containing 20% of a crude antibiotic B—41, which had been obtained by purifying twice by alumina column chromatography the brown oily substance prepared in Example 1, was diluted to the concentrations shown in Table 5, to prepare test solutions. These test solutions were sprayed on to apple leaves infested with about 100 European red mites and, 5 days thereafter, the number of living mites was counted. The results obtained are shown in Table 5, which also shows, for comparison, results obtained with a known compound and with no treatment.

TABLE 5

Concentration	200 p.p.m.	100 p.p.m.	50 p.p.m.
Crude B—41	5/108	14/121	50/115
Reference (Kelthane)	0/97	8/103	56/128
Non-treated		88/102	

(In Table 5, the numerator shows the number of mites before spraying, and the denominator shows the number of living mites at the time of counting.)

Example 4.

Emulsifiable concentrates containing 20% of each in turn of a crude antibiotic B—41 [which had been obtained by purifying by silica gel column chromatography the brown oily substance prepared in Example 1] and a crude $A_1 + A_2 + A_3$ mixture [which had been obtained by subjecting the said crude antibiotic B—41 to column chromatography using a mixed solvent comprising ethyl acetate and benzene (50:50)] were diluted to the concentrations shown in Table 6, to prepare test solutions. These test solutions were sprayed on to orange leaves infested with citrus red mites, and the acaricidal activity (%) after 24 hours was calculated. The results obtained are shown in Table 6, which also shows, for comparison, results obtained with a known compound.

TABLE 6

Concentration	20 p.p.m.	10 p.p.m.	7 p.p.m.	3.3 p.p.m.
Crude B—41		100%		85.5%
Crude $A_1+A_2+A_3$ mixture		100		100
Reference (Kelthane)	90.9		30.8	

Example 5.

Emulsifiable concentrates containing 20% of each in turn of a crude $A_1 + B_1$ mixture [which had been obtained by purifying 3 times by silica gel chromatography (n-hexane: acetone = 70:30) the brown oily substance prepared in Example 1] and a crude $A_2 + A_3 + B_2$ mixture [which had been obtained in the same manner as the former] were diluted to the concentrations shown in Table 7, to prepare test solutions. These test solutions were sprayed onto Chinese cabbages infested with green peach aphids, and the mortality (%) of the aphids after 24 hours was calculated. The results obtained are shown in Table 7, which also shows, for comparison, results obtained with a known compound.

TABLE 7

Concentration	250 p.p.m.	25 p.p.m.	2.5 p.p.m.
Crude A_1+B_1 mixture	100%	92.3%	64.2%
Crude $A_2+A_3+B_2$ mixture	100	89.8	52.8
Reference **	100	73.1	33.1

** *O,O*-Dimethyl-*O*-(2,2-dichlorovinyl)phosphate

Example 6.

First generation rice stem borer eggs were inoculated into rice plants (variety "*Kinmaze*"), which had been planted in 200 cm² pots, and were hatched to allow the larvae to invade the steps. Subsequently a wettable powder containing 40% of a crude antibiotic B—41 (which had been obtained by purifying twice by alumina column chromatography the brown oily substance prepared in Example 1) was diluted to the concentrations shown in Table 8 and then sprayed onto the plants at the rate of 100 cc. per pot. Five days thereafter, the stems were split to examine how many of the larvae were alive and dead, and the mortality (%) of the larvae was calculated. The results obtained are shown in Table 8, which also shows, for comparison, the results obtained with a known compound.

TABLE 8

Concentration	100 p.p.m.	50 p.p.m.
Crude B—41	100%	79.4%
Reference ***	82.1	25.5

*** *O,O*-Diethyl-*O*-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate

As seen in Examples 2 to 6, antibiotic B—41 has excellent insecticidal and acaricidal activity, and is more effective, particularly against mites, than the conventional chemicals.

Procedures for preparing the insecticidal and acaricidal compositions of the present invention are illustrated by Examples 7 to 10, in which all parts are by weight.

Example 7.

10 Parts of a crude antibiotic B—41 (which had been obtained by purifying twice by silica gel column chromatography the brown oily substance prepared in Example 1) were homogeneously mixed with 5 parts of "White Carbon" (a precipitated calcium carbonate of uniform particle size), 50 parts of talc and 35 parts of clay. The resulting mixture was pulverized 3 times by means of an impact type pulverizer and again homogenized to obtain a dust.

Example 8.

40 Parts of the same crude antibiotic B—41 as in Example 7 were homogeneously mixed with 20 parts of "White Carbon", 5 parts of sodium dodecylbenzenesulfonate, 2 parts of polyvinyl alcohol and 33 parts of clay. The resulting mixture was pulverized 3 times by means of an impact type pulverizer and again homogenized to obtain a wettable powder.

Example 9.

20 Parts of the same crude antibiotic B—41 as in Example 7 were homogeneously mixed with 7 parts of polyoxyethylene nonylphenyl ether, 3 parts of calcium dodecylbenzenesulfonate and 70 parts of xylene, and the resulting mixture was filtered to obtain an emulsifiable concentrate.

Example 10.

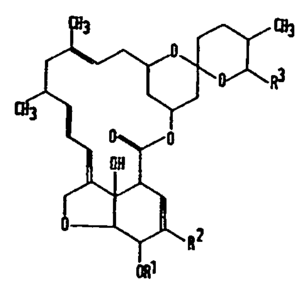
10 Parts of the same crude antibiotic B—41 as in Example 7 were dissolved in 10 parts of xylene. The resulting solution was mixed with 80 parts of machine oil and then filtered to obtain an oil spray.

WHAT WE CLAIM IS:—

1. A process for producing antibiotic substances designated antibiotic B—41, which comprises cultivating the strain *Streptomyces* B—41—146 (Bikokenkinki No. 1438) or an antibiotic B—41-producing mutant thereof in an aqueous nutrient medium therefor and, if desired, recovering the resulting antibiotic B—41 from the fermentation broth.

2. Antibiotic B—41 when produced by the process of claim 1.

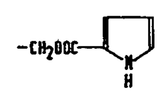
3. Compounds having the formula:



(I)

wherein:

R^1 represents a hydrogen atom, R^2 represents a methyl group or a pyrroyloxymethyl group of formula



and R^3 represents a methyl or ethyl group; or:

R^1 represents a methyl group, R^2 represents a methyl group, and R^3 represents a methyl group or an ethyl group.

4. A compound according to claim 3, wherein R^1 represents a hydrogen atom, R^2 represents a methyl group, and R^3 represents a methyl group.

5. A compound according to claim 3, wherein R^1 represents a hydrogen atom, R^2 represents a methyl group, and R^3 represents an ethyl group.

6. A compound according to claim 3,

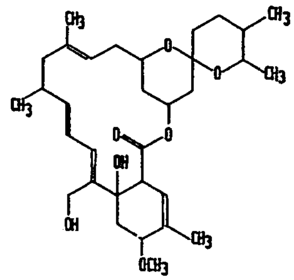
wherein R^1 represents a methyl group, R^2 represents a methyl group, and R^3 represents a methyl group.

7. A compound according to claim 3, wherein R^1 represents a methyl group, R^2 represents a methyl group and R^3 represents an ethyl group.

8. A compound according to claim 3, wherein R^1 represents a hydrogen atom, R^2 represents said pyrroyloxymethyl group, and R^3 represents a methyl group.

9. A compound according to claim 3, wherein R^1 represents a hydrogen atom, R^2 represents said pyrroyloxymethyl group, and R^3 represents an ethyl group.

10. A compound having the formula:



(III)

11. An antibiotic substance designated antibiotic B—41—A_2, obtainable by the cultivation of the strain *Streptomyces* B—41—146 (Bikokenkinki No. 1438), and characterized by the following properties:

(a) when pure it is an amorphous colourless powder
(b) it is sparingly soluble in water, and readily soluble in n-hexane, benzene, acetone ethanol and chloroform;
(c) it has the molecular formula $C_{38}H_{56}O_{10}$;
(d) it has a molecular weight of 672.1 as measured by the osmometric method in acetone, and 672 as measured by mass spectrography;
(e) it has a specific rotation $[\alpha]_D^{20} = +54°$ at a concentration of 5 mg/2 ml and a path length of 10 cm in acetone;
(f) it exhibits no pKa at a pH from 2 to 12;
(g) it exhibits an absorption maximum in the ultraviolet region at 245 mμ;
(h) it exhibits characteristic absorption bands in the infrared region as shown in Figure 11 of the accompanying drawings;
(i) it has the nuclear magnetic resonance spectrum in $(CD_3)_2CO$ shown in Figure 20 of the accompanying drawings;
(j) its mass spectrum measured at 75 eV with an ionization chamber temperature 200° C and a sample temperature of 120—190° C has main peaks at 672, 181, 153 and 151;
(k) it gives the following colour reactions by thin layer chromatography:— yellow to iodine/chloroform, negative to ninhydrin, reddish purple to sulphuric acid, and yellow to potassium permanganate.

12. An antibiotic substance designated antibiotic B—41—B_1, obtainable by the cultivation of the strain *Streptomyces* B—41—146 (Bikokenkinki No. 1438) and characterized by the following properties:

(a) when pure, it is a colourless solid with a melting point of 176—178° C;
(b) It is sparingly soluble in water, and readily soluble in n-hexane, benzene, acetone, ethanol and chloroform;
(c) it has the molecular formula $C_{39}H_{58}O_{10}$;
(d) it has a molecular weight of 629.5 as measured by the osmometric method in acetone, and 686 as measured by mass spectrography;
(e) it has a specific rotation $[\alpha]_D^{20} = +75°$ at a concentration of 5 mg/2 ml and a path length of 10 cm in acetone;
(f) it exhibits no pKa at a pH from 2 to 12;
(g) it exhibits an absorption maximum in the ultraviolet region at 245 mμ;
(h) it exhibits characteristic absorption bands in the infrared region as shown in Figure 14 of the accompanying drawings;
(i) it has the nuclear magnetic resonance spectrum in $(CD_3)_2CO$ shown in Figure 23 of the accompanying drawings;
(j) its mass spectrum measured at 75 eV with an ionization chamber temperature of 200 ° C and a sample temperature of 120—190° C has main peaks at 686, 414, 195, 167, 151 and 125;
(k) it gives the following colour reactions by thin layer chromatography:— yellow to iodine/chloroform, negative to ninhydrin, reddish purple to sulphuric acid, and yellow to potassium permanganate.

13. An insecticidal and/or acaricidal composition comprising at least one antibiotic substance according to any of claims 2 to 12 and an agriculturally acceptable carrier or diluent.

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18 SHEETS This drawing is a reproduction of the Original on a reduced scale
Sheet 1

Fig. 2

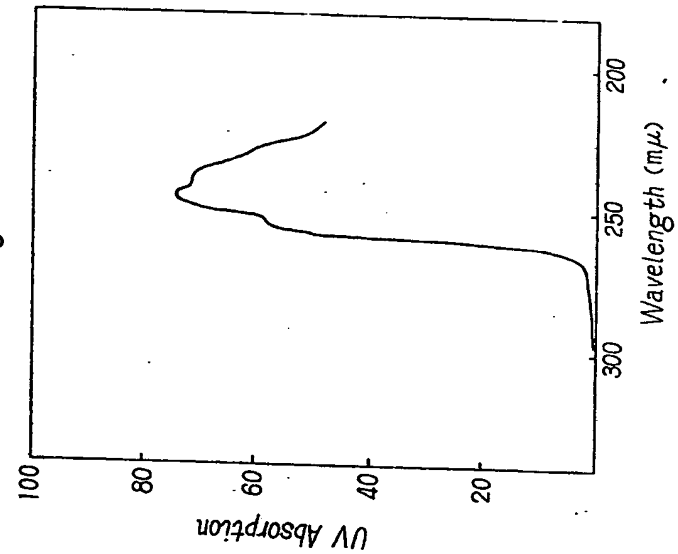
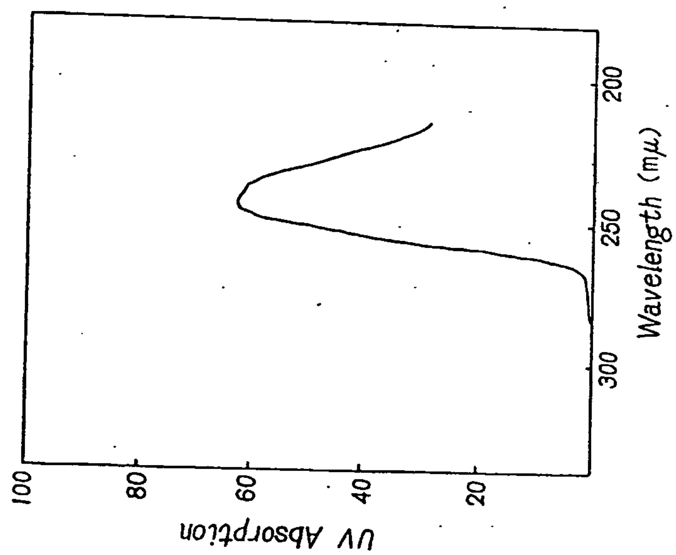


Fig. 1



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Sheet 2

Fig. 4

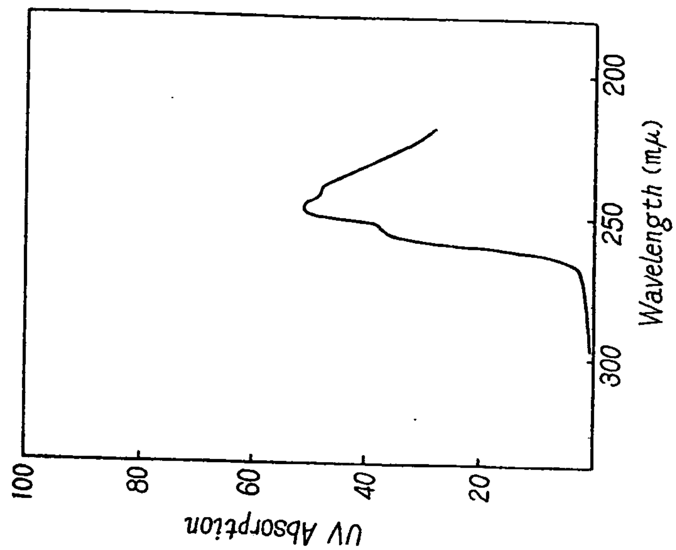
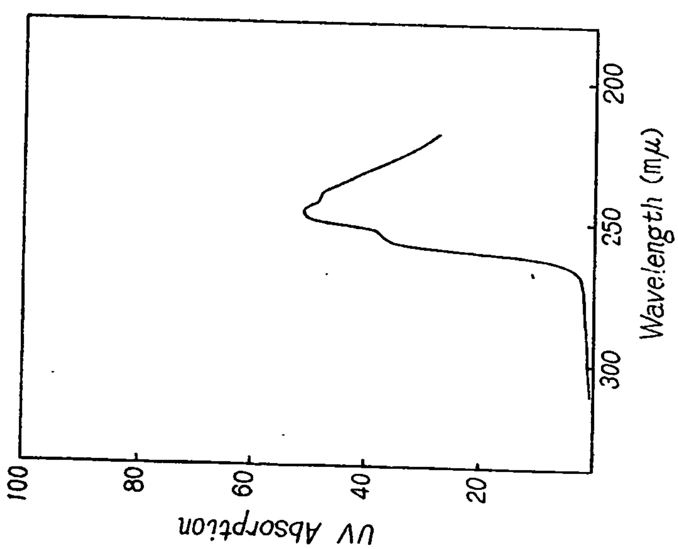


Fig. 3



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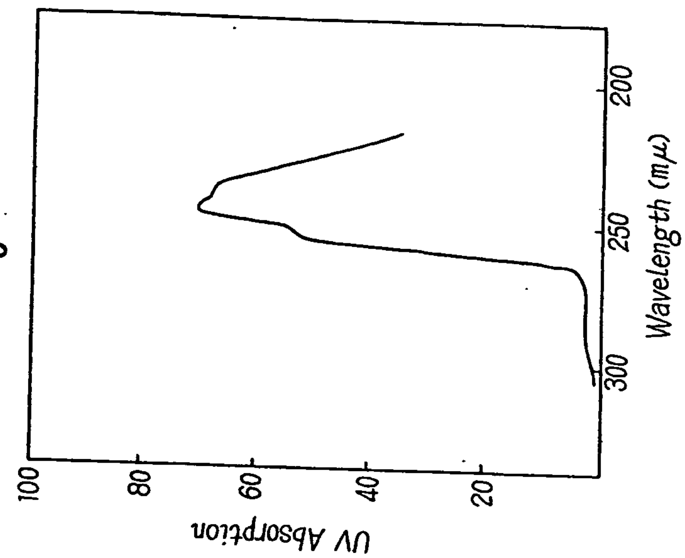
Sheet 3

Fig. 6



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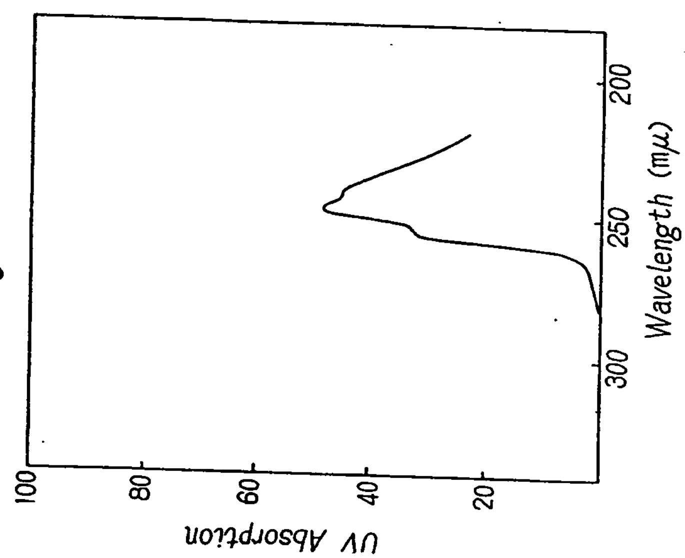
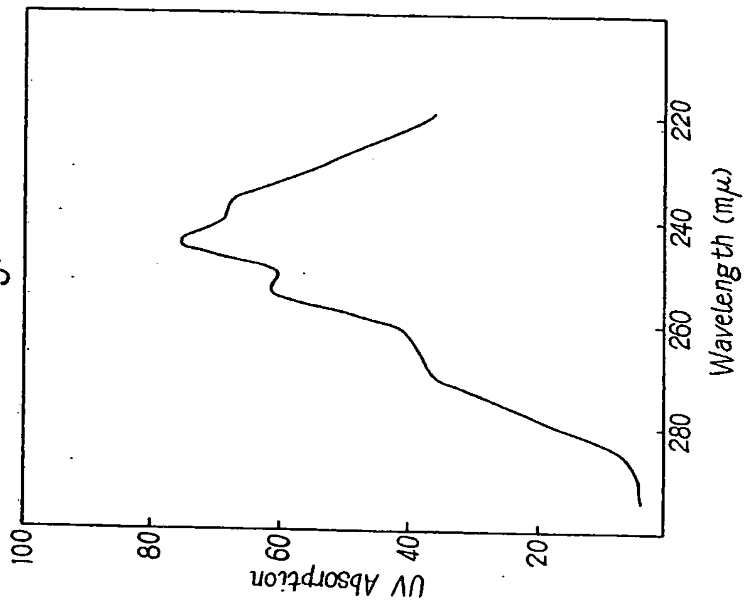


Fig. 8


UV Absorption
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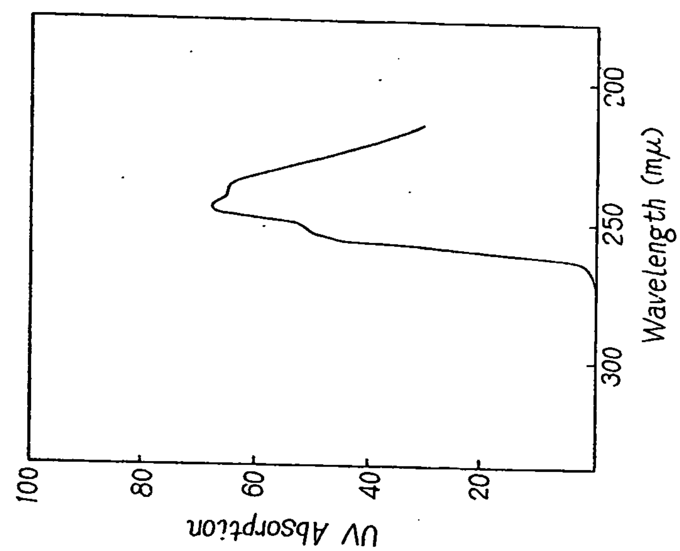
60
40
20
Wavelength (mμ)
220
240
260
280

Fig. 7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Wavelength (mμ)
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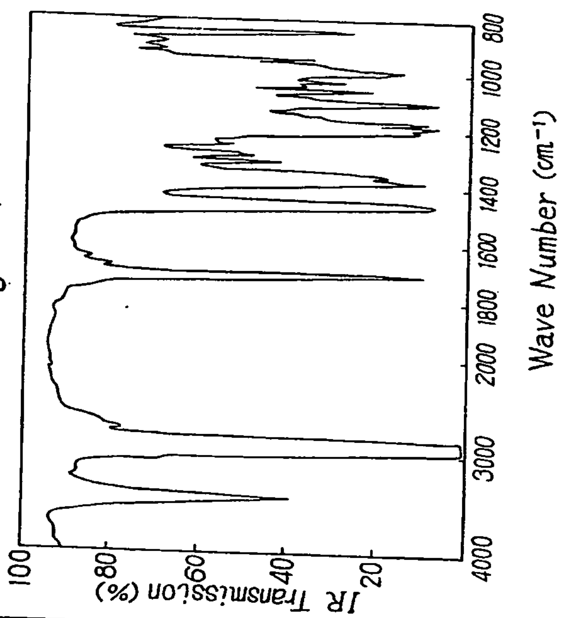
250
300

Fig. 10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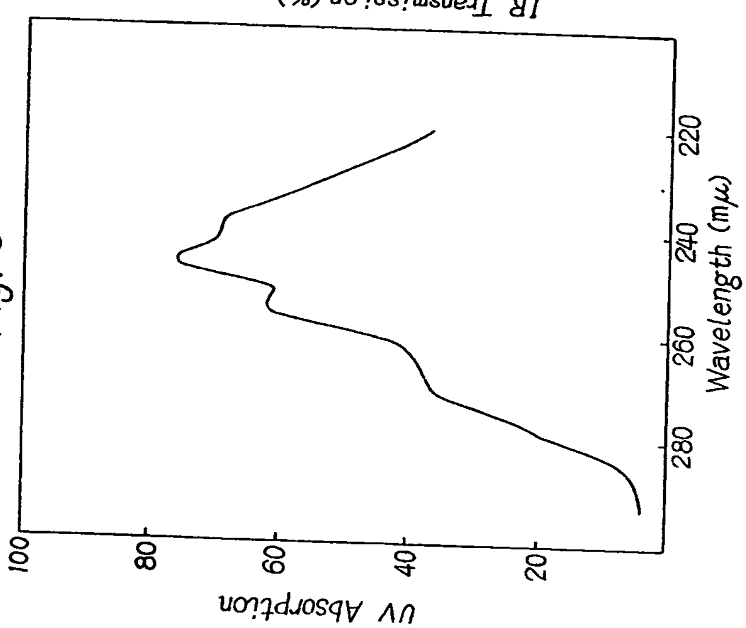
Wave Number (cm⁻¹)

Fig. 9


UV Absorption
100
80
60
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20
280
260
240
220
Wavelength (mμ)

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Sheet 6

Fig.12

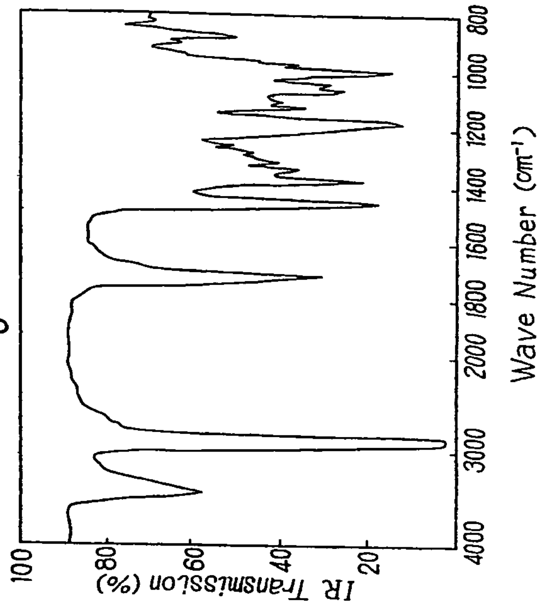


Fig. 11

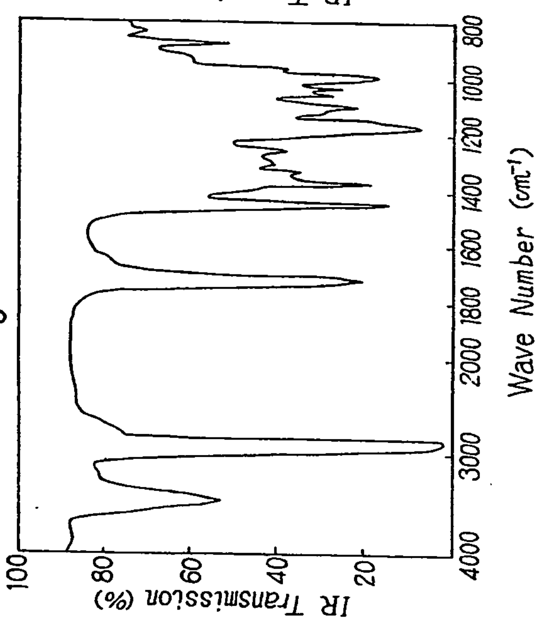
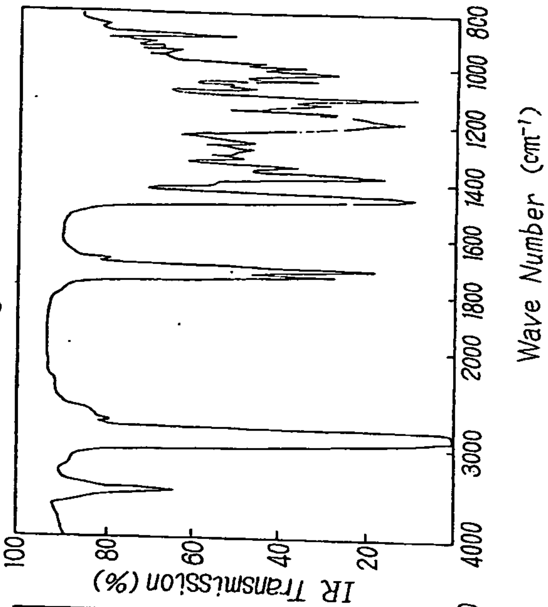


Fig. 14


IR Transmission (%)
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20
4000
3000
2000
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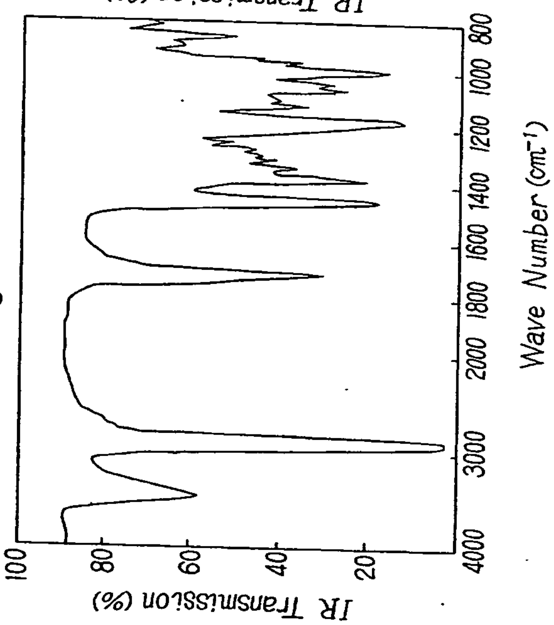
1600
1400
1200
1000
800
Wave Number (cm^{-1})

Fig. 13


IR Transmission (%)
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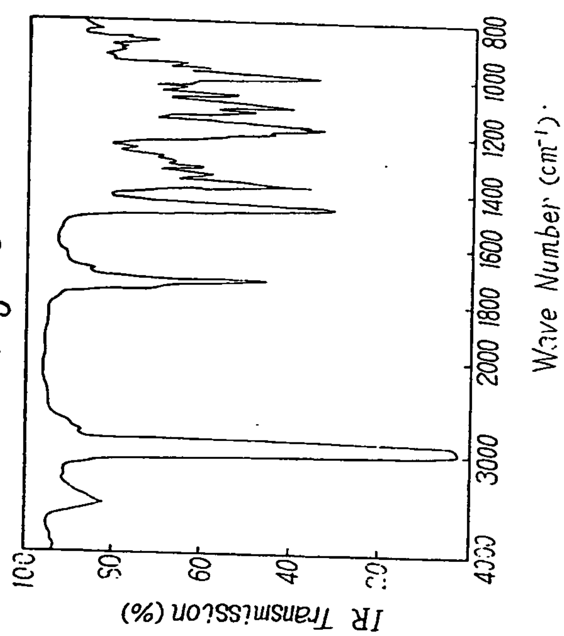
800
Wave Number (cm^{-1})


IR Transmission (%)
Wave Number (cm⁻¹)
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1200
1000
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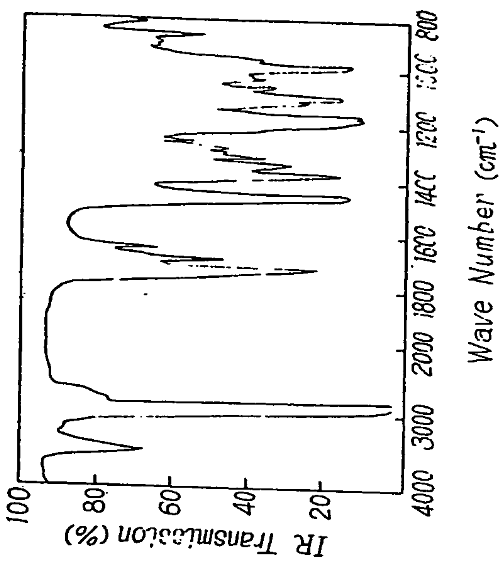
Fig. 15


IR Transmission (%)
Wave Number (cm⁻¹)
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1600
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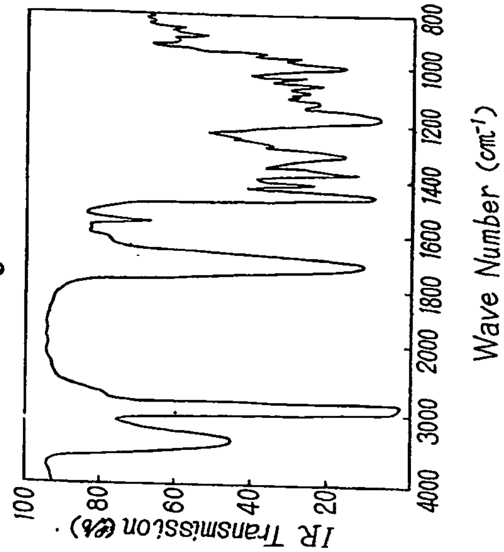
Fig. 16

Fig. 18


IR Transmission (%)
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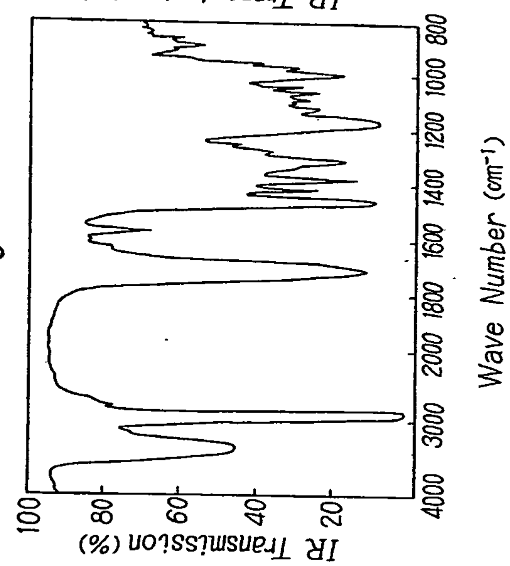
20
4000
3000
2000
1800
1600
1400
1200
1000
800
Wave Number (cm⁻¹)

Fig. 17


IR Transmission (%)
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Wave Number (cm⁻¹)

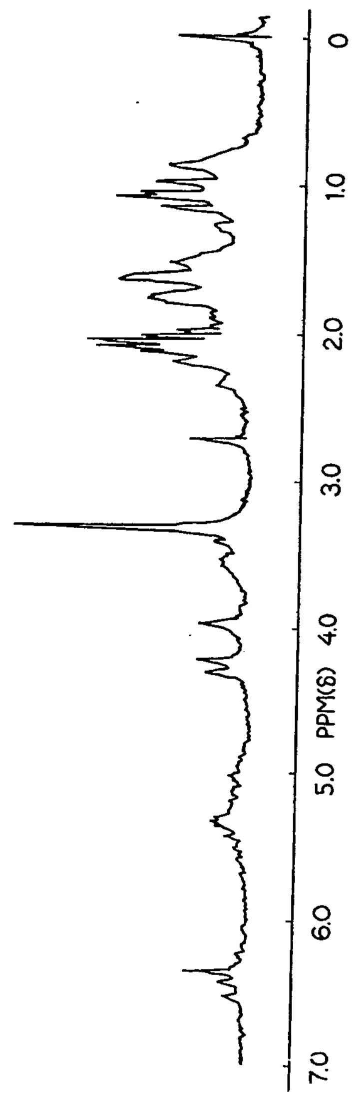

Fig 19

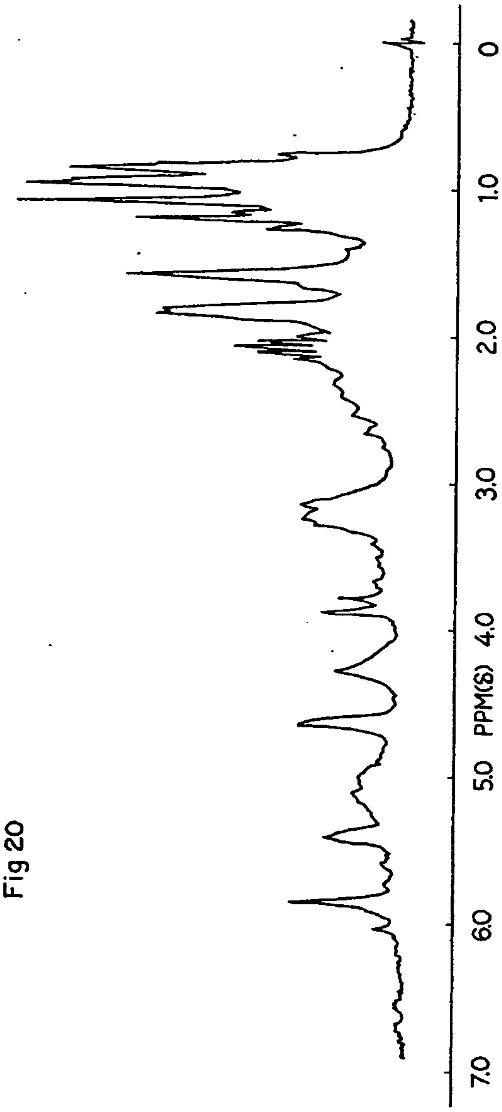

Fig 20

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Sheet 12

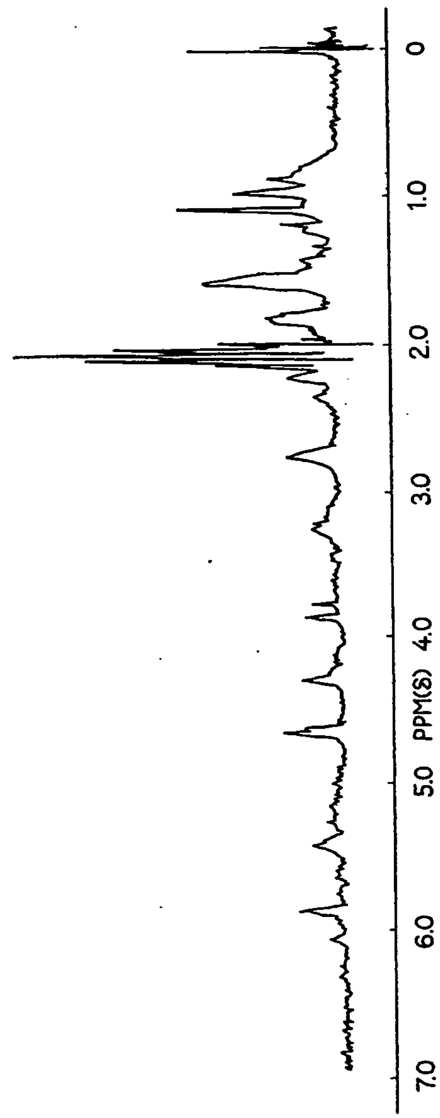

Fig 21

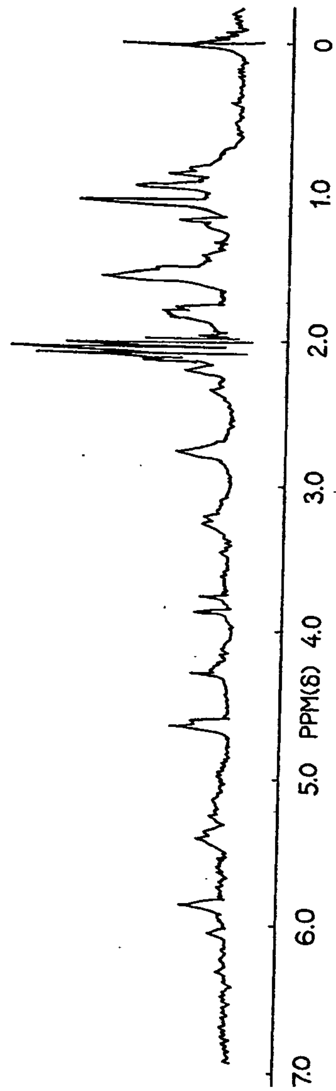

Fig 22

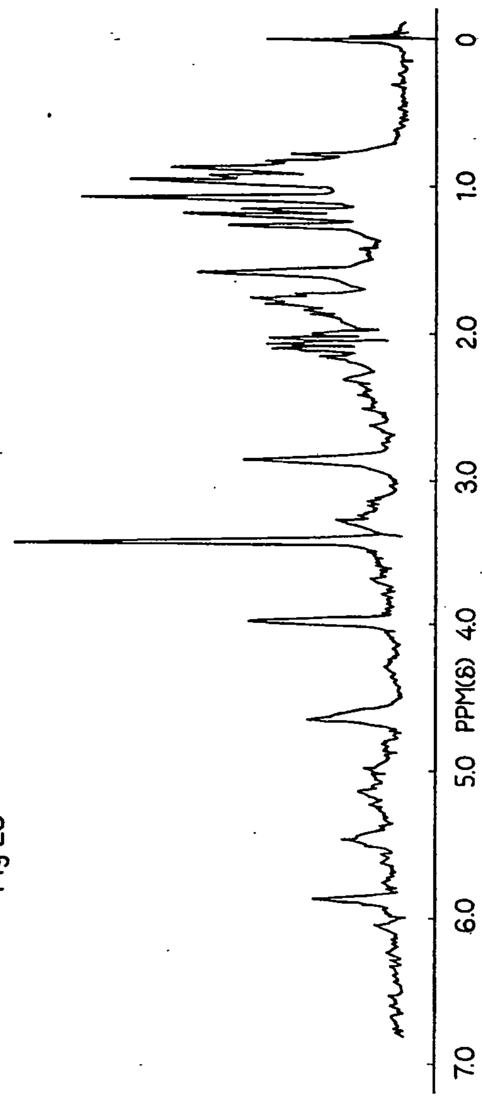

Fig 23

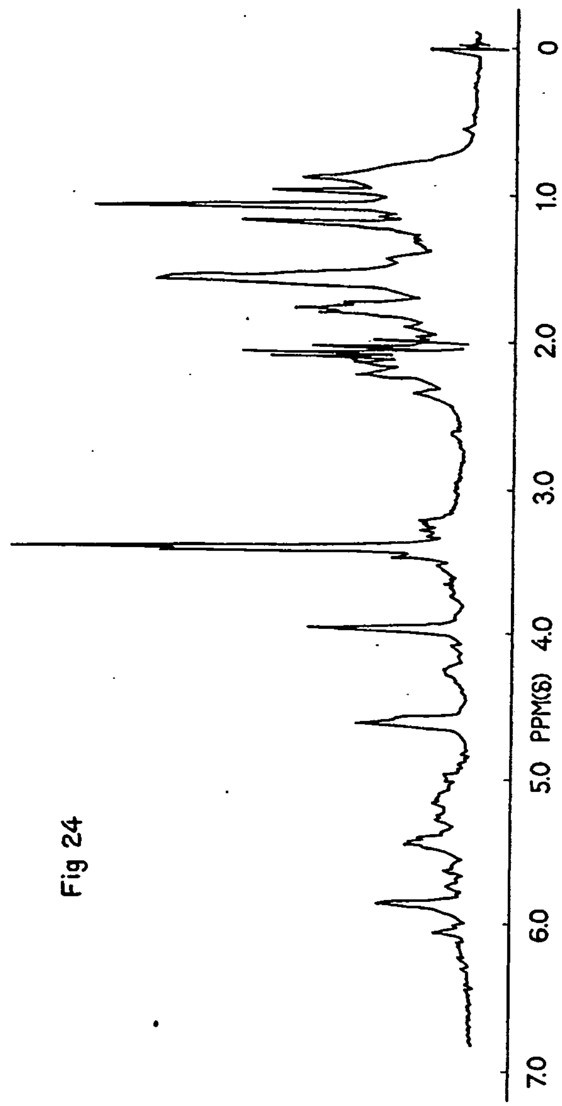

Fig 24

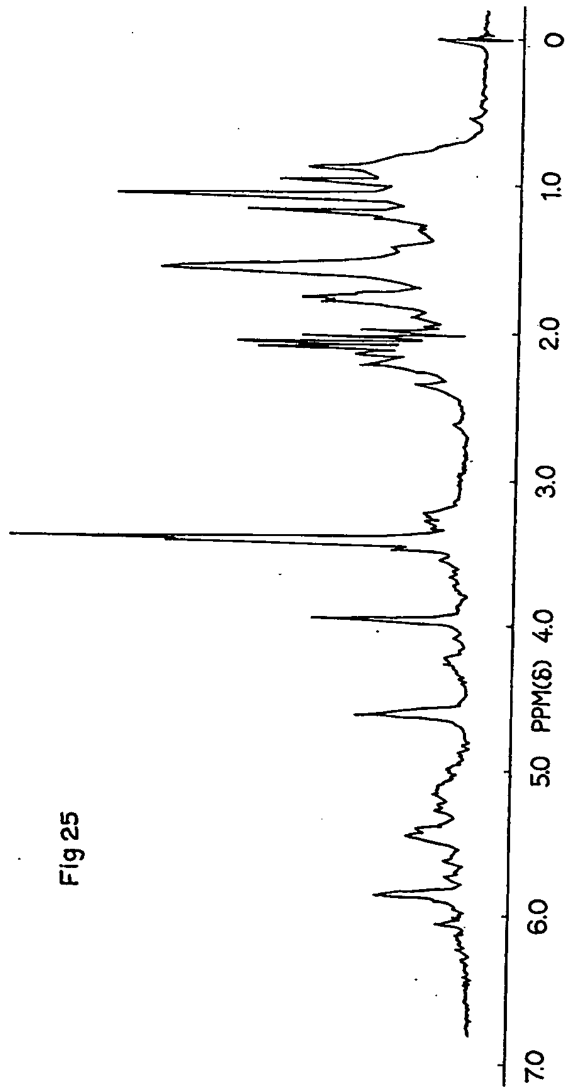

Fig 25

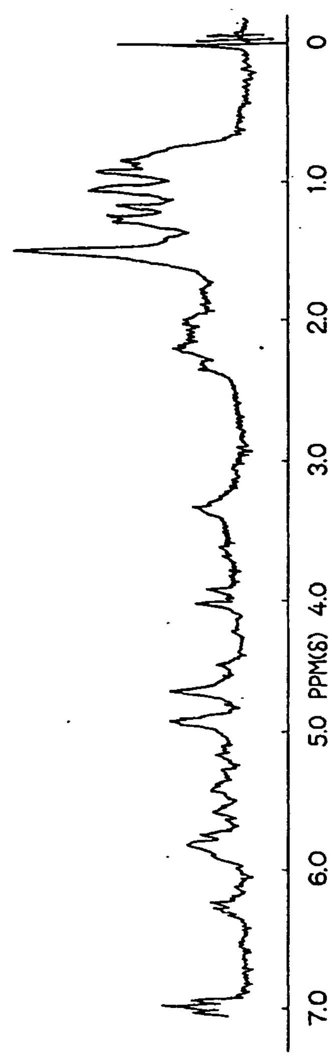

Fig 26

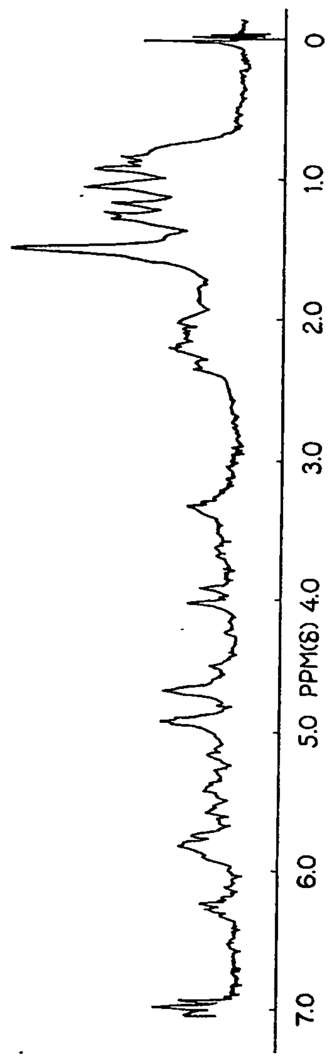

Fig 27

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